determining the stability and rigidity of proteins, shifting the pKa values of buried ionizable residues, and modulating dynamical processes such as folding, catalysis, and proton transfers. Detecting these internal water molecules is sometimes obscured in x-ray crystallography due to positional disorder. We have developed a spectrokinetic assay that accurately detects the presence of a non-coordinated water molecule in the distal heme pocket of myoglobin and in a series of distal pocket mutants, including many where this water molecule is positionally disordered. We also have shown that this water plays a major role in determining the observed bimolecular recombination rate constant. We show that 1) this water molecule modulates the ligand binding dynamics of a series of H64, L29 and V68 mutants; 2) it plays the major role in the observed pH dependence of the CO recombination kinetics between pH 4 and 7 with the protonation of the distal histidine acting as a switch to change water occupancy; and 3) it may also modulate ligand binding dynamics in isolated hemoglobin chains, with the occupancy being larger in the alpha chains. Accurately measuring water occupancy in heme proteins answers crucial questions about water in apolar or slightly polar protein cavities and clarifies the role internal water molecules play in modulating protein function.

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Experimental and Computational Study of the Monomer-Dimer Equilibrium in Dehaloperoxidase from Amphitrite Ornata

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The enzyme dehaloperoxidase (DHP) from the annelid Amphitrite ornata is a unique hemoglobin that functions as a peroxidase, capable of converting 2,4,6-trihalophenols (TBP, TCP, and TFP) into dihalogenated quinones and other products. The DHP protein crystallizes as a dimer. Yet, it was originally characterized as a monomer in solution. We have conducted small angle X-ray scattering (SAXS) in order to probe the monomer-dimer equilibrium in solution. The interest in this area arises from the fact that many hemoglobins are multimers that play an essential role in the cooperativity of oxygen uptake and release. For example, A. ornata possesses a giant hemoglobin (erythrocruorin) like many other marine organisms. Since there are only two known hemoglobin genes (DHP A and DHP B) in A. ornata, it is logical that one or both of these proteins associate with other proteins to form the giant hemoglobin. On the other hand, coelomic DHP does not appear to have a high degree of cooperativity. Moreover, the dimer interface in the X-ray crystal structure of both DHP A and DHP B consists of only 3 amino acid residues. The SAXS data show that the equilibrium for DHP favors the monomer form up to the highest concentrations studied (~ 200 micromolar). However, is there a small amount of the dimer in solution. Thus, it is of interest to apply the known interfaces from study of the X-ray crystal structure to determine which surfaces of DHP may be interacting weakly in solution. We studied the monomer-dimer interface using molecular dynamics (MD) simulations in order to ascertain the relative strength of these interfaces. These results are used to develop a systematic approach to characterization of monomer-multimer equilibria based SAXS and X-ray crystallography data.

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Heme Proteins: The Role of Solvent on the Dynamics of Gates and Portals Revealed by MD Simulations

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In the family of respiratory proteins, hemoglobins and myoglobins have been the first to be crystallized in '50. Despite the availability of 3D structures, issues regarding the microscopic functioning remain open, such as, for instance, the R to T switching mechanism in hemoglobin or the ligand escape process in myoglobin. Due to the relatively small number of residues, myoglobin is the suitable candidate to investigate the more general structure-function paradigm, being defined as the hydrogen atom of biology. In this work, to complement our recent study on the dynamics of internal cavities of myoglobin[1], the effect of solvation on these intrinsic pathways has been explored. In particular, 60ns-long molecular dynamics simulation of horse heart met-myoglobin was further analyzed and the dynamics of waters residing around/inside the protein with average residence times of up to tens of nanoseconds was monitored. Together with the knowledge obtained previously[1], the analysis of solvent revealed that myoglobin has in fact only few stable hydration sites in which a water molecule can stay for time longer than 2 ns. Strikingly, all of these sites are close to protein/solvent portals observed in previous studies focused on the entry/escape and migration of various ligands in myoglobin[2-4].

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3330-Pos

The Effect of Distal Heme Pocket Mutations on the Water Accessible Areas in Myoglobin

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Internal water molecules are important to protein structure and function. A non-coordinated water molecule in the distal pocket of a myoglobin has been shown to be the dominate factor in controlling the binding of CO to the heme active site. We previously developed a method to experimentally measure the entry of internal water into the distal pockets of Mb mutants after photodissociation of CO. In order to better understand what factors control the occupancy of this disordered water in the protein we compared the occupancy with the size of the mutated residue and hydrophobicity. We see little correlation between residue size and water occupancy and a good correlation between water occupancy and hydrophobicity. In order to better understand what factors contribute to internal water occupancy, we further examined how cavity volume and the dynamic behavior of the distal histidine influence water occupancy. Using a computational approach, we calculated the internal volumes of myoglobin cavities for various mutants. We further characterized these cavities by investigating the dynamic behavior of the H64 residue using molecular dynamics. The data show high flexibility of the H64 in the wild type protein suggesting a mechanism by which water is allowed access to the distal cavity. However, in the distal pocket mutants, the H64 can adopt a more stable conformation thereby reducing water access to the cavity. These findings suggest that the flexibility of the distal histidine plays a key role in influencing water access to the distal cavity and the binding affinity for gaseous ligands. In addition, the long range molecular dynamics was used to assess stability of the cavity bound water for the various mutants. The obtained data showed correlation between hydrophobicity and the water residence time in the cavity.

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Ferryl Intermediates in Heme-Based Dioxygenases

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In contrast to the wide spectrum of cytochrome P450 monooxygenases, there are only two heme-based dioxygenases in humans, tryptophan dioxygenase (hTDO) and indoleamine 2,3-dioxygenase (hIDO). hTDO and hIDO catalyze the same oxidative ring cleavage reaction of L-tryptophan (L-Trp) to N-formyl kynurenine (NFK), the initial and rate-limiting step of the kynurenine pathway. Despite immense interest, the mechanism by which the two enzymes execute the dioxygenase reaction remains elusive. Here, we report the first experimental evidence for a key ferryl intermediate of hIDO. It supports a new mechanism, in which the two atoms of dioxygen are inserted into the substrate via a consecutive two-step reaction. This finding introduces a paradigm shift in our understanding of the heme-based dioxygenase chemistry, which was previously believed to proceed via simultaneous incorporation of both atoms of dioxygen into the substrate. The ferryl intermediate is not observable during the hTDO reaction, highlighting the structural differences between the two dioxygenases, as well as the importance of stereoelectronic factors in modulating the reactions.

3332-Pos

Modulation of the Conformation of Cytochrome *c* Oxidase from *paracoccus Denitrificans* by Active-Site Mutations

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We have measured the resonance Raman spectra of the wild-type (wt) and 8 different mutants of cytochrome *c* oxidase from *Paracoccus denitrificans* (pdCcO). Most of the mutants bring about large changes in the binuclear center